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EVALUACIÓN DEL AGUA DE COCIMIENTO DEL PROCESO DE NIXTAMALIZACIÓN APLICANDO TECNOLOGÍAS DE MEMBRANA

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Valorization of Nixtamalization wastewaters (Nejayote) by integrated membrane process

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ABSTRACT

The purpose of this study was to analyze the potentiality of an integrated membrane process for the treatment of Nixtamalization wastewaters (well known as Nejayote). In particular, a sequence of one microfiltration (MF) pre-treatment step followed by two ultrafiltration (UF) processes was investigated on laboratory scale operating in selected process conditions. The performance of selected membranes in terms of productivity, fouling index, and water permeability recovery was evaluated and discussed.

The produced fractions were analyzed for their total soluble solids (TSS), total solids content (TSC), pH, electrical conductivity, turbidity, total polyphenols, total carbohydrates and total organic carbon (TOC). The rejection toward compounds of interest was evaluated. On the basis of experimental results, an integrated membrane process for the treatment of Nejayote was proposed. The conceptual process design permitted to achieve three streams as valuable products: a fraction enriched in carbohydrates, a fraction with high content of calcium components and clear fraction enriched in phenolic compounds. The obtained solution enriched in carbohydrates is of interest for preparing formulations to be used in food industry. Besides, the solution enriched in polyphenols can be used in cosmetic or pharmaceutical applications. Finally, the integrated membrane process used in this study can be used to fractionate Nixtamalization wastewaters as well as avoid the water and environmental pollution by the effluent.

1. Introduction

The maize (*Zea mays*) processing industry generates large amounts of wastewaters due for Nixtamalization process as pretreatment, which is the basis of the commercial methods to produce products such as instant corn flours, tortilla and other corn-based foods (Rojas-Molina et al., 2008, 2009; Gutiérrez et al., 2007). In common process, the maize grains are cooked in a saturated calcium hydroxide solution; the grains are separated from the alkaline solution in order to obtain the Nixtamal product (Valderrama-Bravo et al., 2012). The alkaline solution is well known as Nejayote; within this extract several parts of the grain are lost such as pericarp, germ, dietary fiber and endosperm (Acosta-Estrada et al., 2014). Nixtamalization process is carried out in Mexico, Central America, United States and parts of Europe, a traditional process requires a large volume approximately, 75 L of water is used to process 50 kg of corn kernels, meaning that a similar amount of alkaline wastewater is produced (Niño-Medina et al., 2009). Salmeron-Alcocer et al. (2003) reported that a production of 600 Ton corn/day generates between 1500 and 2000 m^3 of Nejayote, the estimated monthly volume generated in Mexico is about 1.2 millon m^3.

This wastewater contains significant amounts of soluble and insoluble solids consisting of organic and inorganic compounds, the physicochemical properties of Nejayote

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reported by Valderrama-Bravo et al. (2012) are pH (11.39), total solids (2.28%), ash (0.767%), carbohydrates (0.862%), electrical conductivity (4510.12 μS cm^{-1}), total suspended solids (8342.5 mg L^{-1}), turbidity (963.3 NTU) and chemical oxygen demand (40058.14 $mgO_2\ L^{-1}$). However, it has been reported the presence of bioactive compounds in this wastewater as carbohydrates such as arabinoxylans (Niño-Medina et al., 2009; Ayala-Soto et al., 2014), dietary fiber (Acosta-Estrada et al., 2014) and polyphenols (Gutiérrez-Uribe et al., 2010; Lopez-Martinez et al., 2009) such as hydroxycinnamic, p-cumaric, feluric, dehydrodiferulic and dehydrotriferulic acids (Ayala-Soto et al., 2014), but these authors do not describe the method used for separating these chemical compounds from the flowing liquid. Taking into account the characteristics and its chemical composition of this alkaline wastewater, there is strong evidence that the effluent contribute to the water and environmental pollution due to its high biological oxygen demand (BOD) and chemical oxygen demand (COD) (Gutiérrez-Uribe et al., 2010). Nejayote is directly discarded with additional water treatment cost with environmental laws but there are few studies about agro-industrial applications. The extract has been treated to obtain raw material employed as an ingredient to improve boiler foods (Velasco-Martínez et al., 1997), to enrich bread (Acosta-Estrada et al., 2014) or tortillas (Gutiérrez-Uribe et al., 2010).

The water pollution by this wastewater can be avoided trying to recover components from the water stream. Until now, the treatment of Nejayote by membrane operations has been no reported and less has no used as via of recovery of components derived from the wastewater. Microfiltration (MF) and ultrafiltration (UF) processes have been successfully employed for the recovery, purification and concentration of bioactive compounds from different wastewaters such as artichoke wastewaters (Conidi et al., 2014), orange press liquor (Conidi et al., 2012), olive mill wastewaters (Cassano et al., 2013) and winery sludge (Galanakis et al., 2013).

In this work, membrane operations are proposed as an alternative for the treatment of Nejayote. An integrated membrane process based on the use of MF and UF processes was investigated in order to fractionate the Nejayote extract and exploit their high molecular weight components such as suspended solids, calcium components, polyphenols and carbohydrates. In particular, the Nejayote extract was submitted to a preliminary cross-flow MF process in order to recover parts of the grain (fiber, endosperm, pericarp) and suspended solids of high molecular weight. The MF permeate was then processed by two different UF membranes; a concentrated solution in carbohydrates was obtained by 100 kDa membrane, the permeate was then submitted to a 1 kDa membrane, a concentrated solution rich in calcium components was produced and finally, a clear solution enriched in polyphenols was obtained. The rejection toward compounds of interest was evaluated.

The performance of the selected membranes was also determined in terms of productivity, fouling index and recovery of hydraulic membrane permeability.

2. Materials and methods

2.1. Solutions and reactants

2.1.1. Preparing the extracts

To prepare Nejayote, Nixtamalization was carried out as follows: total of 5 kg of corn was cooked during 38 min at 92 °C in a solution prepared with 50 g of calcium hydroxide (Nixtacal-Mexico) dissolved in 10 L of water. Then corn kernels were rested during 12 h; the Nejayote (cooking liquor) was drained by decantation. The extract was then stored at −17 °C until used.

2.2. Experimental set-up and procedures for the treatment of Nejayote by membranes

2.2.1. Selection of the feed flow rate condition in MF and UF membranes

In order to find the optimal feed flow rate of Nejayote in the membranes. The influence of the feed flow rate on permeate flux at 25 °C at optimal transmembrane pressure was analyzed. Same experiments were carried out for all membranes according to the procedure described by Cassano et al. (2007), the maximum permeate fluxes were found at 58 L h^{-1} for all membranes.

2.2.2. Microfiltration treatment

Nejayote was submitted to a preliminary MF process in order to eliminate suspended solids and high molecular weight compounds to reduce fouling phenomenon in the next UF processes. MF experiments were performed using laboratory unit, the MF unit was equipped with a polysulfone hollow fiber membrane module (Amersham Biosciences Corp., Model CFP-1-E-4A, USA) with nominal pore size of 0.2 μm.

The MF system was operated at a TMP of 1.3 ± 0.03 bar, at an axial flow rate (Q_f) of 58 L h^{-1} and a temperature of 25 ± 1 °C according to the batch concentration mode (recycling the retentate stream and collecting separately the permeate). Permeate fluxes were measured up to volume reduction factor (VRF) of 7. The VRF was defined as the ratio between the initial feed volume and the volume of resulting retentate according to the following Eq. (1) (Garcia-Castello et al., 2011):

$$\mathrm{VRF} = \frac{V_F}{V_R} = 1 + \frac{V_P}{V_R} \quad (1)$$

where V_F, V_p and V_r are the volumes of feed, permeate and retentate, respectively.

2.2.3. Ultrafiltration treatment

Permeate obtained from MF was submitted to a UF step. The UF step was performed using a laboratory unit with feed tank, and peristaltic pump, a water bath at constant temperature was used to avoid significant increasing in the fluid temperature, a manometer and pressure-regulating valve. The UF unit was equipped with two different polysulfone hollow fiber membranes (Amersham Biosciences Corp., Model UFP-100-E-4A & UFP-1-E-4A, USA) of 100 and 1 kDa. The specifications of the membranes are reported in Table 1. UF experiments were carried out according to the recirculation configuration at an operating temperature of 25 ± 1 °C at different transmembrane pressures (0.3, 0.6, 1.0, 1.3 and 1.7 bar) in order to find the optimal pressure that provides the limiting flux for carrying out batch concentration configuration.

The experimental runs were performed according to the batch concentration mode up to a final VRF of 6, at TMP of 1.3 bar and VRF of 5, at TMP of 1.7 bar for 100 and 1 kDa membranes, respectively. The system was operated at a temperature of 25 ± 1 °C. The feed flow rate was fixed at 58 L h^{-1}.

For MF and UF steps, the permeate flux was measured gravimetrically as the change of permeate weight with time

Table 1 – Specifications of the membranes used for the treatment of Nejayote.

Membranes	MF	UF	UF
Manufacturer	Amersham Biosciences	Amersham Biosciences	Amersham Biosciences
Membrane type	CFP-1-E-4A	UFP-100-E-4A	UFP-1-E-4A
Nominal pore size (μm)	0.2	–	–
MWCO (Da)	–	100,000	1000
Membrane surface area (cm^2)	420	420	420
Membrane material	Polysulfone	Polysulfone	Polysulfone
Configuration	Hollow fiber	Hollow fiber	Hollow fiber
pH operating range	2–14	2–14	2–14
Temperature range (°C)	2–80	2–50	2–50
Transmembrane pressure operating range (bar)	0.4–3	0.4–3	0.4–3

using a digital balance, the permeate flux of productivity was expressed in $kg\,m^{-2}\,h^{-1}$. Samples of feed, permeate and retentate taken during experimental runs were kept in the freezer (−17 °C) until analysis.

2.3. *Membrane properties: water permeability, fouling index and cleaning efficiency*

According to the Darcy's law (Strathmann et al., 2006):

$$Lp = \frac{Jv}{\Delta P} \tag{2}$$

the water permeability (Lp) of each membrane was determined by the slope of the straight line obtained plotting the water flux (Jv) values, measured in fixed conditions of temperature (25 °C) against the applied transmembrane pressure (Δp).

The fouling index (I_f), expressed as a percentage drop in water permeability, was performed by measuring the water permeability before and after treatment of Nejayote, according the following equation:

$$I_f = \left(1 - \frac{Lp_1}{Lp_0}\right) \cdot 100 \tag{3}$$

where Lp_0 and Lp_1 are the water permeabilities measured before and after treatment of Nejayote, respectively.

After treatment of Nejayote, MF and UF membranes were submitted to an enzymatic cleaning with Ultrasil 67 (Ecolab, Minnesota, USA) at 0.5% (v/v) (60 min, 55 °C). At the end of each cleaning procedures membranes were rinsed with tap water for 30 min and water permeability was measured again.

Cleaning efficiency was evaluated by using the flux recovery (FR,%) method (Chen et al., 2006) according to the following equation:

$$F_R = \left(\frac{Lp_2}{Lp_0}\right) \cdot 100 \tag{4}$$

where Lp_2 is the water permeability measured after enzymatic cleaning.

2.4. *Physico-chemical parameters evaluated on Nejayote*

Feed, permeate and retentate samples coming from different membrane operations were analyzed in terms of Total soluble solids (TSS), polyphenols, total carbohydrates (as sugars), electrical conductivity, pH, turbidity, total solids content (TSC) and total organic carbon (TOC).

2.4.1. *Polyphenols*

Total phenols were estimated colorimetrically by using the Folin–Ciocalteu method (Singleton et al., 1999). The method is based on the reduction of tungstate and/or molybdate in the Folin–Ciocalteau reagent by phenols in alkaline medium resulting in a blue colored product ($\lambda_{max} = 756\,nm$), results were expressed as mg gallic acid L^{-1}.

2.4.2. *Total carbohydrates*

The total carbohydrates as sugars were estimated colorimetrically by using Dubois method (Dubois et al., 1956); results were expressed as mg glucose mL^{-1}.

2.4.3. *Total soluble solids (TSS)*

TSS, in terms of °Brix was measured by using a hand refractometer PAL-3 (Atago Co., Tokyo, Japan) with a scale range of 0–50 °Brix.

2.4.4. *Electrical conductivity*

Electrical conductivity was measured by 510 model pH meter (OAKTON, LA, USA), using a special electrode to determine electrical conductivity. It was calibrated with KCl 0.1 M solution; the electrical conductivity was expressed as $\mu Siemens\,cm^{-1}$ ($\mu S\,cm^{-1}$).

2.4.5. *pH*

pH was measured by 510 model pH meter (OAKTON, LA, USA). It was calibrated with buffer solutions of pH 4 and 7.

2.4.6. *Turbidity*

Turbidity in samples was analyzed with HI93703 model Microprocessor Turbidity-meter (Hanna instruments, Romania); it was calibrated with patron solutions 0–1000 NTUs.

2.4.7. *Total solids content (TSC)*

Total solids content in samples was measured by the following equation:

$$\%\text{Total solids content}: \quad 100 - \%\text{moisture} \tag{5}$$

where the moisture was determined by method 925.09 (AOAC, 1998).

2.4.8. *Total organic carbon (TOC)*

TOC was determined employing by Shimadzu equipment (Model TOC-5000) expressed as mg mL^{-1} (Akechi et al., 2008, Patent No. WO2008114410A1).

2.4.9. *Statistical analysis*

All measurements on the physicochemical parameters were performed in triplicate. Results were expressed as

Table 2 – Physico-chemical composition of Nejayote before and after clarification with MF membrane.

Sample	TSS (°Brix)	TSC (%)	Turbidity (NTU)	pH	Conductivity (μS cm^{-1})	Total carbohydrates (mg mL^{-1})	Polyphenols (mg gallic acid L^{-1})	TOC (mg L^{-1})
Feed	1.53 ± 0.05^b	1.34 ± 0.05^b	538.09 ± 12.12^b	13.48 ± 0.00^b	2424.00 ± 5.29^b	3.47 ± 0.09^b	1190.74 ± 23.62^b	2984.10 ± 0.94^b
Permeate	1.3 ± 0.00^c	1.02 ± 0.06^c	146.51 ± 3.73^c	13.42 ± 0.00^c	1896.66 ± 0.57^c	2.96 ± 0.20^c	964.81 ± 20.47^c	2845.5 ± 0.77^c
Retentate	3.4 ± 0.10^a	1.66 ± 0.12^a	1767.8 ± 5.55^a	13.62 ± 0.00^a	2792.33 ± 2.51^a	4.51 ± 0.04^a	1350.92 ± 43.59^a	3620.4 ± 0.91^a

Data represents the means ± deviation standard with triplicate for each test. Different superscript letters in the column indicate statistical significance ($p < 0.05$) according to the Tukey's least significant difference test.

mean ± standard deviation (SD), and one-way analysis of variance (ANOVA) were performed using SAS statistical software (V6.0, SAS Institute S.A de C.V., Mexico). Tukey's multiple range test was used to compare the means. Differences among the means of $p < 0.05$ were considered significant.

2.5. *Rejection toward each component by used membranes*

To determine the rejection (R_i) of specific compound by the membranes was determined as the following equation (Cissé et al., 2011):

$$R_i = \left(1 - \frac{Cp_i}{Cf_i}\right) \cdot 100 \qquad (6)$$

where Cp_i and Cf_i are the concentration of specific compound in permeate and feed, respectively.

3. Results and discussion

3.1. *Nejayote composition*

The general composition of Nejayote submitted to the MF treatment is reported in Table 2. The pH value of the MF feed was of about 13.48, higher value than 11.39 reported by Valderrama-Bravo et al. (2012). On the contrary, the author obtained higher values on turbidity (963 NTU) and electrical conductivity (4510 μS cm^{-1}) compared of 538 NTU and 2424.0 μS cm^{-1} that our study reported. The TOC values of Nejayote were of about 2984.10 mg mL^{-1} higher values than reported by that study, which was reported as biochemical oxygen demand. Basically, even when our study followed the basic preparation instruction to carry out a common Nixtamalization process, the physicochemical composition of Nejayote depends of the lime quality, type of grain, grain quality, cooking time, and some other parameters as were reported by Gutiérrez-Uribe et al. (2010) and Ruiz-Gutiérrez et al. (2010).

The content of polyphenols on Nejayote was of about 1190.74 mg mL^{-1} higher content than reported as the content of low molecular weight polyphenols on other wastewaters as artichoke wastewater of 516.7 mg mL^{-1} (Conidi et al., 2014) and olive mill wastewaters of 211.80 mg mL^{-1} (Garcia-Castello et al., 2010). Similar content compared to our study was observed by Cassano et al. (2013), 1409 mg mL^{-1} in terms of total polyphenols in olive mill wastewaters. On the other hand, the content of total carbohydrates in Nejayote extract was of about 3.47 mg mL^{-1} (expressed as glucose), a lower value than other wastewaters. Nevertheless, Ayala-Soto et al. (2014) confirmed that the sugars composition in Nejayote is basically related to arabinoxylans, which are derived from hydrolyzed maize fiber by the alkaline treatment, these types of compounds are of interest due to their functionality on baking industry (Acosta-Estrada et al., 2014). Then, due to these

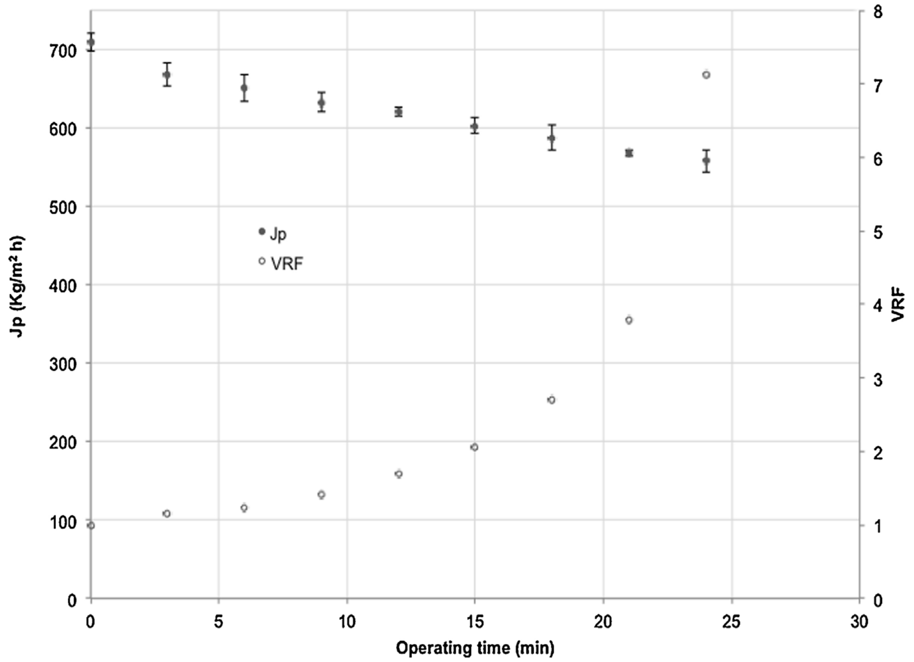

Fig. 1 – Microfiltration of Nejayote. Time course of permeate flux and VRF (operating conditions: TMP: 1.3 bar, Q_f: 58 L h^{-1}, *T*: 25 ± 1 °C).

Table 3 – Water permeability, fouling index and cleaning efficiency of MF and UF membranes.

Membrane type	Lp_0 ($L\,m^{-2}\,h^{-1}\,bar^{-1}$)	Lp_1 ($L\,m^{-2}\,h^{-1}\,bar^{-1}$)	Lp_2 ($L\,m^{-2}\,h^{-1}\,bar^{-1}$)	If (%)	CE (%)
MF 0.2 μm	751.66	581.13	750.36	22.68	99.82
UF 100 kDa	61.82	7.69	61.39	87.55	99.29
UF 1 kDa	2.32	1.45	2.32	37.50	100.00

characteristics Nejayote can be considered a by-product that represents a very useful source of high added value compounds (polyphenols and carbohydrates) of potential interest in food additives and nutraceutical.

3.2. *MF membrane performance on Nejayote*

Fig. 1 shows the productivity of the hollow fiber MF membrane in terms of kg of permeate produced per unit area and time ($kg\,m^{-2}\,h^{-1}$) in the treatment of Nejayote, in selecting operating conditions up to VRF of 7. The initial permeate flux was of about 700 $kg\,m^{-2}\,h^{-1}$; it decreased during the process by increasing the VRF due to the concentration polarization phenomenon, fouling and concentration of solutes in retentate as suspended solids, parts of grain (Acosta-Estrada et al., 2014) and insoluble solids (as insoluble calcium) (Pflugfelder et al., 1988). However, the initial permeate flux decreased slowly and continued until to reach a steady-state value higher than 500 $kg\,m^{-2}\,h^{-1}$; resulting in a time run of the separation process by batch concentration configuration about 24 min. This decline could be attributed to the continually removal of the macromolecules on the membrane surface and the high pore size of the membrane.

After enzymatic cleaning, the initial water permeability (751.66 $L\,m^{-2}\,h^{-1}\,bar^{-1}$) of the MF membrane was almost completely recovered, the enzymatic cleaning step permitted to recover 99.82% of the initial permeability of the membrane (Table 3). A low fouling index in the membrane (22.68%) was determined using Nejayote extract. Normally, the low fouling on membrane surface by the treatment of solutions rich in calcium is attributed to the formation and migration of precipitates (as $Ca(OH)_2$) that produce their free movement and easy removal (Ayala-Bribiesca et al., 2006). However, taken into account the incomplete recovery of the initial water permeability of the MF membrane by the treatment of Nejayote, from an internal pore blocking to a cake formation can be supposed due to the presence of large pore size can permit the penetration of particles in the internal structure of the membrane causing an irreversible fouling (Cassano et al., 2007).

3.3. *UF membrane performance on Nejayote*

The MF permeate was processed by using two different UF membranes. Fig. 2 shows the productivity of the hollow fiber UF membrane (100 kDa) in terms $kg\,m^{-2}\,h^{-1}$ in the treatment of Nejayote permeate from MF step, in selecting operating conditions up to VRF of 6. The initial permeate flux was of about 22 $kg\,m^{-2}\,h^{-1}$; it decreased rapidly within the first 10 min and continued to decrease until to reach a productivity higher than 10 $kg\,m^{-2}\,h^{-1}$. The initial permeate flux continually decreasing during the process by increasing the VRF due to the concentration polarization phenomenon, fouling and concentration of solutes in retentate associated to the separation of the suspended solids and carbohydrates. After enzymatic cleaning, the measure of the permeability (61.82 $L\,m^{-2}\,h^{-1}\,bar^{-1}$) on the 100 kDa membrane was almost completely recovered, the membrane presented a high fouling index (87.55%). The enzymatic-cleaning step permitted to recover 99.29% of the initial permeability of the membrane (Table 3), in this process also can be supposed from an internal

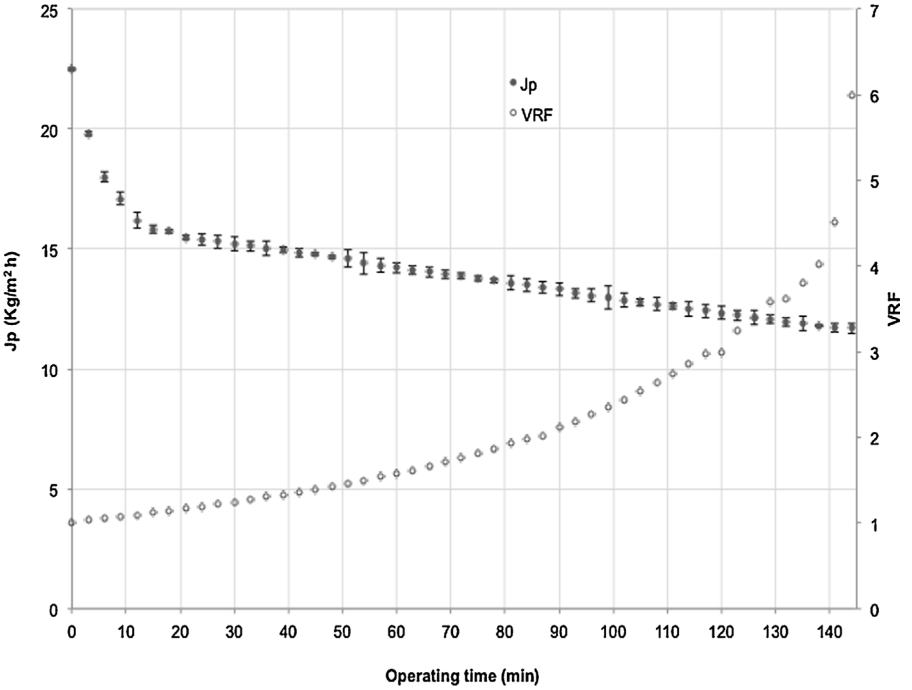

Fig. 2 – Ultrafiltration (100 kDa) of Nejayote. Time course of permeate flux and VRF (operating conditions: TMP: 1.3 bar, Q_f: 58 $L\,h^{-1}$, T: 25 ± 1 °C).

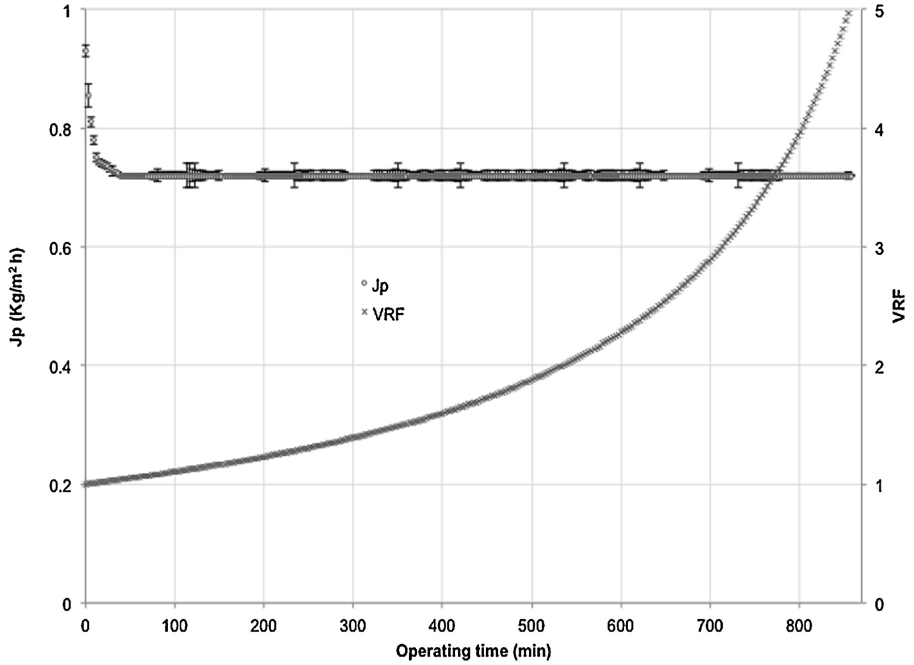

Fig. 3 – Ultrafiltration (1 kDa) of Nejayote. Time course of permeate flux and VRF (operating conditions: TMP: 1.7 bar, Q_f: 58 L h^{-1}, T: 25 ± 1 °C).

pore blocking to a cake formation in the membrane by the particles causing an irreversible fouling as well as incomplete recovery of the initial permeability (Cassano et al., 2007).

Furthermore, Fig. 3 shows the productivity of the hollow fiber UF (1 kDa) membrane with Nejayote permeate from UF (100 kDa) step in selecting operating conditions up to VRF of 5. The initial permeate flux was of about 0.9 kg m^{-2} h^{-1}; it decreased during the process by increasing the VRF due to the concentration of solutes in retentate as soluble solids (soluble calcium) (Pflugfelder et al., 1988) and polarization phenomenon. The initial permeate flux decreased immediately within the first 15–20 min and continued to decrease until to reach a steady-state value higher than 0.7 kg m^{-2} h^{-1}; this decline can be attributed to the rejection of the soluble components in the aqueous system. After enzymatic cleaning, the initial water permeability (2.32 L m^{-2} h^{-1} bar^{-1}) of the UF membrane was totally recovered, the enzymatic cleaning step permitted to recover 100.00% of the initial permeability of the membrane (Table 3), the complete recovery of the initial water permeability can be supported by a fouling: partial pore blocking where the particles can block/unblock the pore of the membrane and it can be removed easily (Cassano et al., 2007). In addition, low fouling index in the membrane about 37.5% was determined. In this membrane the rejection of soluble

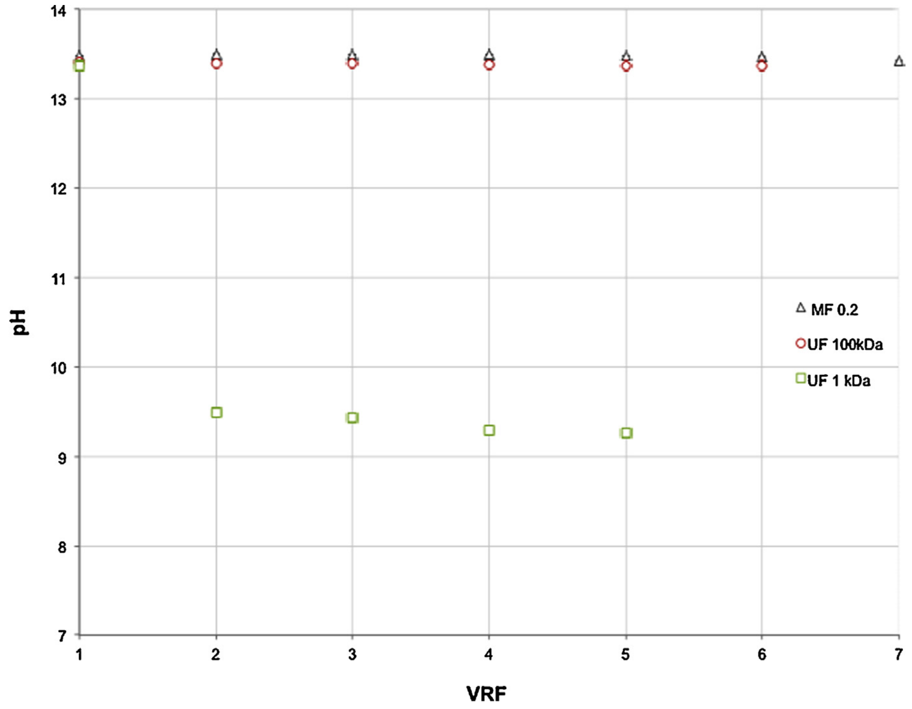

Fig. 4 – pH evolution in permeate fractions of MF and UF membranes as a function of VRF.

calcium can be produced the same phenomenon described by Ayala-Bribiesca et al. (2006). Moreover, the wastewater treated is an extract with a high alkalinity degree and it can act as a chemical cleaning in membranes, a variety of alkalis are used in the food industry as cleaning agents such as hydroxides, phosphates, sulphates and carbonates usually of sodium and phosphorus but the calcium-complexes can support the hydrolysis and solubility of specific compounds as carbohydrates, fats, or proteins (D'Souza and Mawson, 2005).

Basically, the incomplete recovery of the initial permeability of some membranes is attributed to an irreversible component of fouling; the polyphenols are able to be absorbed on the surface of the membranes as reported by Saleh et al. (2006) and Sotto et al. (2013). Nevertheless, it can see that the membranes are beneficiaries in terms of recovery of the initial permeability due to the extremely properties on pH of the Nejayote.

3.4. *Influence of integrated membrane process on Nejayote physico-chemical composition*

pH analysis in Nejayote samples coming from the integrated membrane process revealed a decreased on this property. Fig. 4 shows the pH evolution in permeate fractions from MF and UF membranes on function of VRF. Basically, MF and (100 kDa) UF membranes do not show interesting changes on the property, minimal changes were found after treatment from these steps. In the first step, permeate with pH of 13.42 was obtained from the extract with pH of 13.48. Similarly, permeate obtained from the (100 kDa) UF membrane presented a pH value of 13.37 (see Table 4). Even, the retentates from these steps do not show a considerable increase in pH by the concentration of the components related of this property. In addition, Fig. 4 shows immediate changes on 1 kDa permeate, pH values started to decrease at VRF of 2 and during the evolution of the process the values continuously decreased up to pH values around 9.0. A reduction of this property can be attributed of the rejection of dissolved soluble calcium by this membrane (Pflugfelder et al., 1988). The extremely pH of the Nejayote can be useful in terms of productivity in the membranes due to there is strong evidence that high pH values increase the permeate fluxes, this phenomenon was observed by Akdemir and Ozer (2009) in the treatment of alkaline wastewater using polysulfone UF membranes.

In addition, Fig. 5 shows the electrical conductivity behavior of the extract over integrated membrane process on function of VRF; the decreasing of the pH values by removal of the calcium components was confirmed. The decline of these components on the Nejayote influenced on the changes of pH property directly associated to the electrical conductivity of the stream. High electrical conductivity of the calcium hydroxide in aqueous solution was demonstrated by Safavi and Nakayama (2000), and it is directly related to the concentration of dissolved solids in water. UF (1 kDa) membrane demonstrated as the suitable membrane to removal the soluble calcium in this wastewater. The initial electrical conductivity of Nejayote was about 2424.00 μS cm^{-1} (see Table 2) and the final permeate from 1 kDa membrane was 874.33 μS cm^{-1} (see Table 4), it means, a 64% of this property was decreased in the permeate stream and increased in retentate samples (3924.33 μS cm^{-1}).

Fig. 6 summarizes rejection values obtained for Turbidity, TSS and TSC. Basically, MF and UF (100 kDa) membranes are the suitable membranes to removal turbidity on Nejayote,

Table 4 – Physico-chemical composition of Nejayote in different samples coming from the UF treatment.

Membrane type	Sample	TSS (°Brix)	TSC (%)	Turbidity (NTU)	pH	Conductivity (μS cm^{-1})	Total carbohydrates (mg mL^{-1})	Polyphenols (mg gallic acid L^{-1})	TOC (mg L^{-1})
UF (100 kDa)	Feed	1.27 ± 0.01^{b}	1.02 ± 0.06^{b}	145.6 ± 1.21^{b}	13.41 ± 0.00^{b}	1896.66 ± 0.57^{c}	3.02 ± 0.05^{b}	970.37 ± 16.03^{b}	2784.60 ± 1.06^{b}
	Permeate	0.8 ± 0.00^{c}	0.79 ± 0.00^{c}	5.6 ± 0.64^{d}	13.37 ± 0.00^{c}	1558.33 ± 0.57^{d}	1.09 ± 0.03^{d}	950.00 ± 19.44^{e}	1889.16 ± 0.38^{d}
	Retentate	3.06 ± 0.05^{a}	2.88 ± 0.00^{a}	1379.0 ± 24.24^{a}	13.44 ± 0.00^{a}	3758.33 ± 2.08^{b}	7.72 ± 0.03^{a}	958.33 ± 12.10^{c}	6972.00 ± 2.38^{a}
UF (1 kDa)	Feed	0.8 ± 0.00^{c}	0.79 ± 0.00^{c}	5.44 ± 0.84^{e}	13.37 ± 0.00^{c}	1558.33 ± 0.57^{d}	1.08 ± 0.00^{e}	970.37 ± 16.03^{b}	1908.48 ± 0.35^{c}
	Permeate	n.d^{e}	0.17 ± 0.00^{e}	3.78 ± 0.00^{f}	9.00 ± 0.01^{f}	874.33 ± 3.05^{e}	0.26 ± 0.00^{f}	951.85 ± 6.99^{d}	381.99 ± 0.03^{f}
	Retentate	1.03 ± 0.05^{d}	0.74 ± 0.00^{d}	31.43 ± 3.09^{c}	13.28 ± 0.01^{d}	3924.33 ± 5.13^{a}	1.40 ± 0.01^{c}	986.11 ± 10.01^{a}	1744.89 ± 0.05^{e}

Data represents the means ± deviation standard with triplicate for each test. Different superscript letters in the column indicate statistical significance ($p < 0.05$) according to the Tukey's least significant difference test.

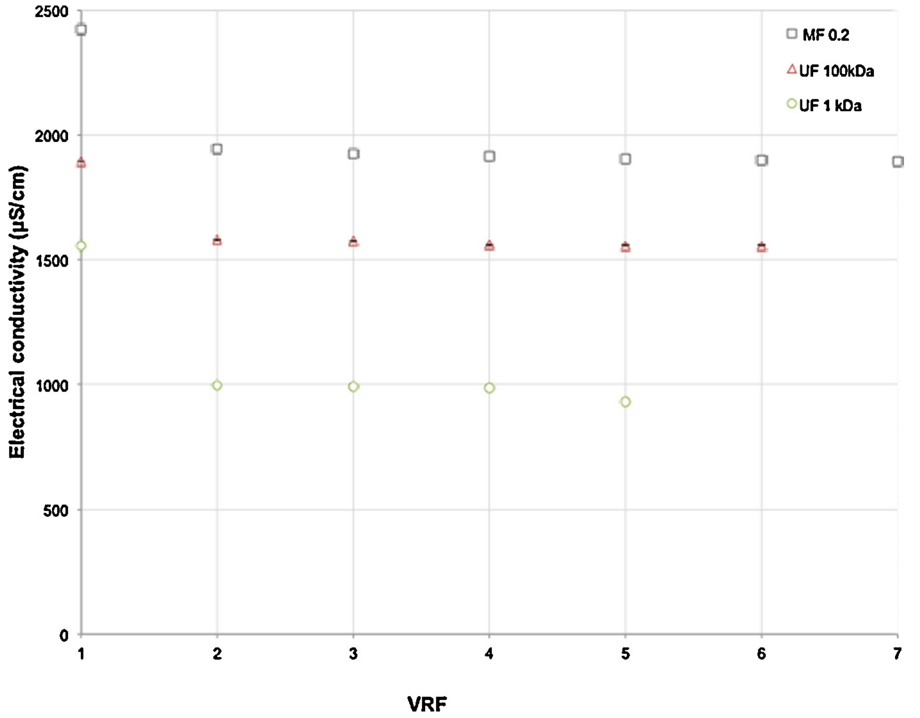

Fig. 5 – Electrical conductivity evolutions in permeate fractions of MF and UF membranes as a function of VRF.

rejection values more than 70% and 95% were reached, respectively. The feed samples in MF process presented turbidity values around 538 NTU (see Table 2) and the permeate samples presented turbidity values about of 146 NTU, the turbidity removed by MF membrane is associated to the suspended solids, parts of the grain and insoluble components due to the decline on TSC content (from 1.34 to 1.03%) and on TSS (from 1.53 to 1.3 °Brix) which represent higher rejection values than 22% and 35%, respectively (Fig. 6). On the contrary, feed samples in 100 kDa membrane reported 145.6 NTU and permeates obtained from this membrane presented 5.6 NTU (Table 4). Basically, the removal mechanism of these macromolecules by MF module with large pore size is governed by the separation via sieving mechanism, based on their molecular weight where the retention is absolutely carried out by the barrier as well as the fouling mechanism produced by the macromolecules (Todisco et al., 1996; Galanakis, 2014). The turbidity removed by 100 kDa membrane can be related to the total carbohydrates because the components were rejected more than 64% of them (obtaining a concentrated retentate with 7.72 mg mL^{-1}), it means, 3.02 mg mL^{-1} was decreased up to 1.09 mg mL^{-1} in permeate; in case of carbohydrates

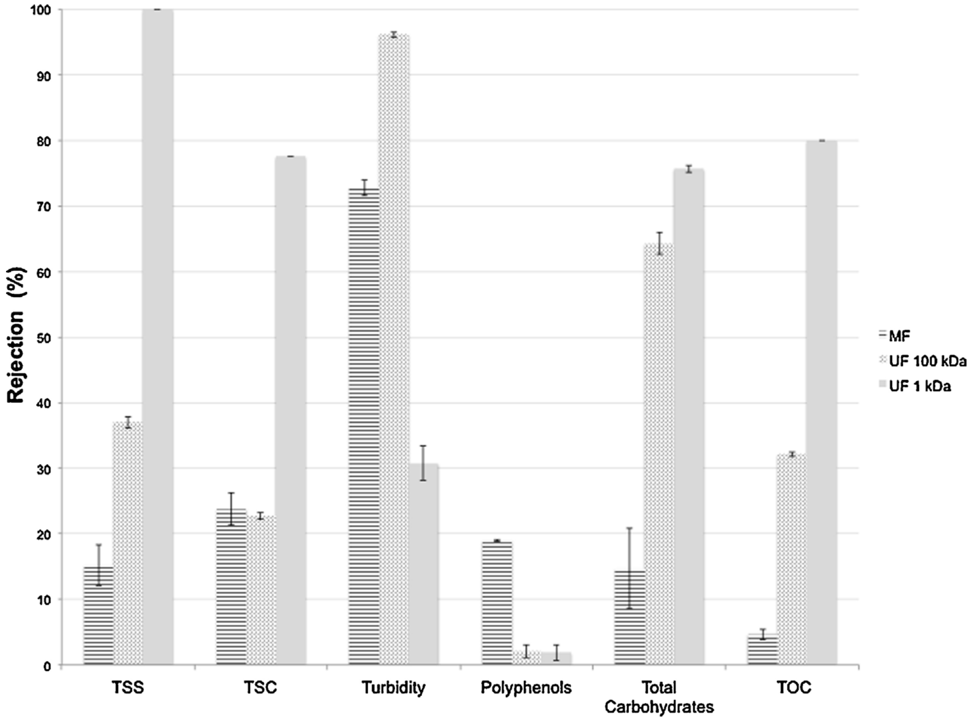

Fig. 6 – Rejections of MF and UF membranes toward TSS, TSC, turbidity, polyphenols, total carbohydrates and TOC.

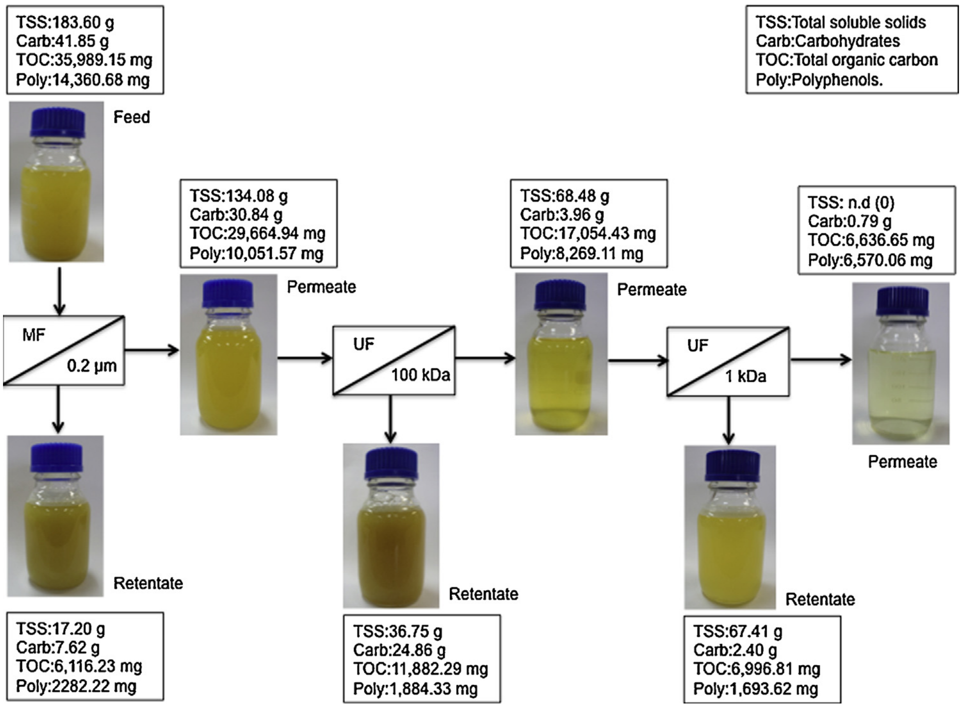

Fig. 7 – Permeate and retentate samples obtained from the treatment of Nejayote by integrated membrane process.

retention by UF modules with wide membrane pore (100–50 kDa), the membrane material and the type of macro and micro-molecules can play an important role where the separation process seems to be simple in theory since membranes are able to separate compounds via sieving mechanism, based on their molecular weight. However, this is not the case in practice, as the asymmetric manufacture of membrane pores does not always reflect a narrow range of molecular weight cut-off (MWCO). The effect of the latest parameter attenuates when the solubility of the solutes and the hydrophobicity of the membrane surface are incorporated; thereby, MWCO is not an absolute barrier for the separation of macro- and micro-molecules such as sugars, carbohydrates, hydrolyzed dietary fibers where all these factors can produce that micromolecules can be retained and increase the total retention (Galanakis, 2014). The 1 kDa membrane also can be used to removal low molecular weight carbohydrates because more than 75% no rejected by 100 kDa membrane. However, the major concentration in carbohydrates was achieved by 100 kDa membrane. In addition, the major amounts of components rejected by 1 kDa membrane were soluble compounds such as total soluble solids and soluble calcium, Table 4 shows the TSC and TSS content in feed samples, 0.79% and 0.8 °Brix, respectively, the final permeate from this membrane presented values about of 0.17% in TSC and TSS was No determined (value close to 0), the rejections of these components were of 100% and higher value than 70%, respectively (Fig. 6). High rejections on TSS and TSC (associated to the calcium compounds and Ca^{++} ions) can be carried out by 1 kDa membrane due to these narrower membrane pores are considered as a Nanofiltration barrier (Galanakis, 2014). The separation is conducted by the solute solubility, polarity resistances and membrane hydrophobicity where last facts produce several process restrictions. Conidi et al. (2012) reported higher rejection of Ca^{++} from orange press liquor using 1 kDa membrane.

Concerning about total polyphenols content, Fig. 6 shows the rejection of the membranes toward total polyphenols. Rejection values were about 18%, 2% and 1.9% for MF, 100 and 1 kDa membranes, respectively. It is clear that the used membranes cannot reject considerable amount of these components due to their molecular weight; however, the membranes rejected significant polyphenols content due to the concentration polarization phenomenon, fouling (associated to a cake formation) and also by the concentration of solutes in retentate (Cassano et al., 2007). In addition, high rejection in polyphenols by MF membrane can be attributed to the precipitation of a calcium-organic complexes that are co-precipitated or absorbed occurs at higher pH values (Al-Amoudi and Lovitt, 2007). These products may be contributing to the rejection of polyphenols and it can be considered them as a barrier as well as contribute to the cake formation. It can see that 951 mg L^{-1} (Table 4) from 1190 mg L^{-1} of the initial content of feed sample from integrated membrane process was recovered in permeate from 1 kDa, it means, a clarified solution rich in polyphenols was obtained. The low molecular weight of polyphenols no allowed to be rejected by 1 kDa membrane; Cassano et al. (2013) also observed a low rejection of polyphenols using 1 kDa membrane; so the same, the polyphenols were recovered in permeate stream.

Finally, Fig. 6 summarizes rejection values obtained for TOC in different steps of the integrated membrane process, rejection values of 4.6, 32.1 and 79.9% were reached with the treatment of Nejayote by membrane operations, the final permeate from the system (1 kDa permeate) obtained about 380 mg L^{-1} from 2984 mg L^{-1} (Tables 2 and 4) in MF feed samples. Basically, the final organic load of the final permeate from 1 kDa membrane could be associated to the total polyphenols recovered from all integrated membrane system.

3.5. *Proposal of an integrated membrane process for the treatment of Nejayote*

Fig. 7 shows a picture of permeate and retentate samples obtained in the treatment of Nejoyate with the selected

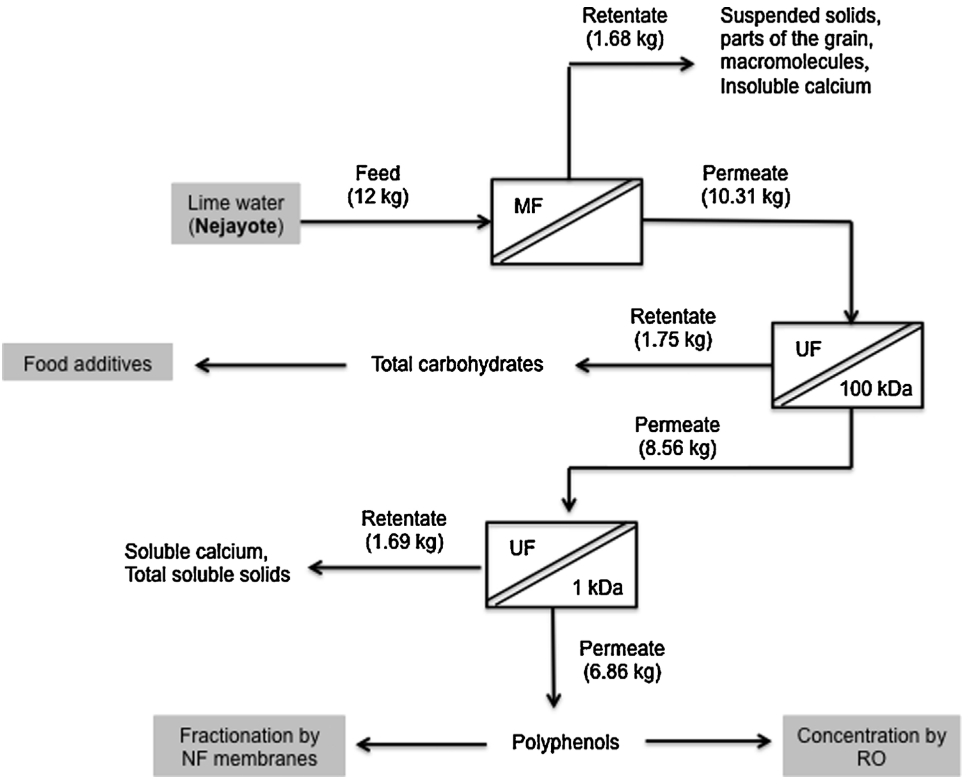

Fig. 8 – Proposed process scheme for the valorization of Nejayote based on MF and UF operations.

membranes. The 1 kDa permeate resulted a clear solution with high content of phenolic compounds suggesting a potential use for several applications as cosmetic, food, and pharmaceutical formulations. i.e. polyphenols are widely used by pharmaceutical, cosmetic and food sectors (Garcia-Castello et al., 2010) due to their properties such as anti-inflamatory, antimicrobic and antioxidant activity as well as inhibition of oxidative damage and the radical elimination (Ranalli et al., 2003; Bisignano et al., 1999; Obied et al., 2005). These compounds are usually synthesized by chemical methods that are responsible of their high price as well as their low yields obtained (Conidi et al., 2014). On the other hand, an integrated membrane process can be used for the fractionation by NF membranes or concentration using Reverse osmosis. In case of total carbohydrates identified as arabinoxylans could be used in food additive applications for their common utilization on bakery industry (Acosta-Estrada et al., 2014); and calcium components can be reused in the Nixtamalization treatment of maize as calcium hydrated (Valderrama-Bravo et al., 2012).

On the basis of experimental results, a process scheme for recovering water, phenolic compounds, total carbohydrates and calcium components from Nejayote is suggested (Fig. 8). Integrated membrane processes reported in literature for selective fractionation of polyphenols from different wastewaters such as Olive Mill Wastewaters (Cassano et al., 2013; Garcia-Castello et al., 2010; Paraskeva et al., 2007) and Artichoke wastewaters (Conidi et al., 2014) include several membrane processes as final step to produce a permeate fraction (water) with quality characteristics that make it possible to be discharged in aquatic system. Nevertheless, based on these results obtained and with the proposed membrane process without additional step give a strong evidence that the treatment contribute to avoid the water and environmental pollution by the recovery of chemical components from the extract.

4. Conclusions

The investigated process based on MF process followed by two UF processes, permits to operate a separation of Nejayote where was separated organic and inorganic compounds of different molecular weight. Basically, four different fractions are produced:

1. A concentrated solution rich in suspended solids, parts of the grain and macromolecules (MF retentate): this fraction could be used as feed on livestock or can be used as carbon source to a biotechnological process in order to produce biogas, bioethanol or other type of biotechnological products.
2. A concentrated solution rich in carbohydrates (100 kDa UF retentate): this fraction can be used to food additives.
3. A concentrated solution containing components of soluble calcium (1 kDa UF retentate): this fraction can be reused in the Nixtamalization process of maize.
4. A clear solution enriched in polyphenols (1 kDa UF permeate): this fraction could be used in cosmetic, food, and pharmaceutical industries as well as liquid formulations after fractionation by NF membranes or concentration by reverse osmosis.

Finally, the treatment of Nejayote by membrane process is a real possibility to avoid the water pollution through fractionation of this stream. In addition, the treatment represents an environmental solution in order to decrease the biochemical oxygen demand.

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References

Acosta-Estrada, B.A., Lazo-Vélez, M.A., Nava-Valdez, Y., Gutiérrez-Uribe, J.A., Serna-Saldívar, S.O., 2014. Improvement of dietary fiber, ferulic acid and calcium contents in pan bread enriched with nejayote food additive from white maize (*Zea mays*). J. Cereal Sci. 60, 264–269.

Akdemir, O., Ozer, A., 2009. Investigation of two ultrafiltration membranes for treatment of olive mil wastewaters. Desalination 249, 660–666.

Akechi, M., Abe, H., Fujiyama, Y., 2008. Patent No. WO2008114410A1, In Shimatzu.

Al-Amoudi, A., Lovitt, R.W., 2007. Fouling strategies and the cleaning system of NF membranes and factor affecting cleaning efficiency. J. Membr. Sci. 303, 4–28.

AOAC, 1998. Official Methods of Analysis. Official Method 925.09, 16th ed. Association of Official Analytical Chemists, Maryland, USA.

Ayala-Bribiesca, E., Araya-Farias, M., Pourcelly, G., Bazinet, L., 2006. Effect of concentrate solution pH and mineral composition of a whey protein diluate solution on membrane fouling formation during conventional electrodialysis. J. Membr. Sci. 280, 790–801.

Ayala-Soto, F.E., Serna-Salivar, S.O., García-Lara, S., Pérez-Carrillo, E., 2014. Hydroxycinnamic acids, sugar composition and antioxidant capacity of arabinoxylans extracted from different maize fiber sources. Food Hydrocolloids 35, 471–475.

Bisignano, G., Tomaino, A., Lo Cascio, R., Crisafi, G., Uccella, N., Sajia, A., 1999. On the in-vitro antimicrobial activity of oleuropeína and hydroxytyrosol. J. Pharm. Pharmacol. 51, 971–974.

Cassano, A., Marchio, M., Drioli, E., 2007. Clarification of blood orange juice by ultrafiltration: analyses of operating parameters, membrane fouling and juice quality. Desalination 212, 15–27.

Cassano, A., Conidi, C., Giorno, L., Drioli, E., 2013. Fractionation of olive mill wastewaters by membrane separation techniques. J. Hazard. Mater. 248–249, 185–193.

Cissé, M., Vaillant, F., Pallet, D., Dornier, M., 2011. Selecting ultrafiltration and nanofiltration membranes to concentrate anthocyanins from roselle extract (*Hibiscus sabdariffa* L.). Food Res. Int. 44, 2607–2614.

Chen, V., Li, H., Tan, D.S., Petrus, H.B., 2006. Cleaning strategies for membrane fouled with proteins mixtures. Desalination 200, 198–200.

Conidi, C., Cassano, A., Drioli, E., 2012. Recovery of phenolic compounds from orange press liquor by nanofiltration. Food Bioprod. Process. 90, 867–874.

Conidi, C., Cassano, A., Garcia-Castello, E., 2014. Valorization of artichoke wastewaters by integrated membrane process. Water Res. 48, 363–374.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.

D'Souza, N.M., Mawson, A.J., 2005. Membrane cleaning in the dairy industry: a review. Crit. Rev. Food Sci. Nutr. 45, 125–134.

Galanakis, C.M., Markouli, E., Gekas, V., 2013. Recovery and fractionation of different phenolic classes from winery sludge using ultrafiltration. Sep. Purif. Technol. 107, 245–251.

Galanakis, C.M., 2014. Separation of functional macromolecules and micromolecules: from ultrafiltration to the border of nanofiltration. Trends Food Sci. Tecnol., http://dx.doi.org/10.1016/j.tifs.2014.11.005.

Garcia-Castello, E.M., Mayor, L., Chorques, S., Arguelles, A., Vidal-Brotons, D., Gras, M.L., 2011. Reverse osmosis concentration of press liquor from orange juice solid wastes: flux decline mechanisms. J. Food Eng. 106, 199–205.

Garcia-Castello, E., Cassano, A., Criscuoli, A., Conidi, C., Drioli, E., 2010. Recovery and concentration of polyphenols from olive mill wastewaters by integrated membrane system. Water Res. 44, 3883–3892.

Gutiérrez, E., Rojas-Molina, I., Pons-Hernández, J.L., Guzmán, H., Aguas-Ángel, B., Arenas, J., Fernández, P., Palacios-Fonseca, A., Herrera, G., Rodríguez, M.E., 2007. Study of calcium ion diffusion in nixtamalized quality protein maize as a function of cooking temperature. Cereal Chem. 84, 186–194.

Gutiérrez-Uribe, J., Rojas-García, C., García-Lara, S., Serna-Saldivar, S., 2010. Phytochemical analysis of wastewater (nejayote) obtained after lime-cooking of different types of maize processed into masa for tortillas. J. Cereal Sci. 52, 410–416.

Lopez-Martinez, L.X., Oliart-Ros, R.M., Valerio-Alfaro, G., Lee, C.H., Parkin, K.L., Garcia, H.S., 2009. Antioxidant activity, phenolic compounds and anthocyanins content of eighteen strains of Mexican maize. LWT – Food Sci. Technol. 42, 1187–1192.

Niño-Medina, G., Carvajal-Millán, E., Lizardi, J., Rascon-Chu, A., Marquez-Escalante, J., Gardea, A., Martínez-López, A., Guerrero, V., 2009. Maize processing wastewater arabinoxylans: gelling capability and cross-linking content. Food Chem. 115, 1286–1290.

Obied, H.K., Allen, M.S., Bedgood, D.R., Prenzler, P.D., Robards, K., Stockmann, R., 2005. Bioactivity and analysis of biophenols recovered from olive mill waste. J. Agric. Food Chem. 53, 823–837.

Paraskeva, C.A., Papadakis, V.G., Tsarouchi, E., Kanellopoulou, D.G., Koutsoukos, P.G., 2007. Membrane processing for olive mill wastewater fractionation. Desalination 213, 218–229.

Pflugfelder, R.L., Roony, L.W., Waniska, R.D., 1988. Dry matter losses in comercial corn masa production. Cereal Chem. 65 (2), 127–132.

Ranalli, A., Lucera, L., Contento, S., 2003. Antioxidizing potency of phenol compounds in olive mill wastewater. J. Agric. Food Chem. 51, 7636–7641.

Rojas-Molina, I., Gutiérrez, E., Cortés-Acevedo, M.E., Falcón, A., Bressani, R., Rojas, A., Ibarra, C., Pons-Hernández, J.L., Guzmán-Maldonado, S.H., Cornejo-Villegas, A., Rodríguez, M.E., 2008. Analysis of quality protein changes in nixtamalized QPM flours as a function of the steeping time. Cereal Chem. 85, 409–416.

Rojas-Molina, I., Gutiérrez, E., Rojas, A., Cortés-Álvarez, M., Campos-Solís, L., Hernández-Urbiola, M., Arjona, J.L., Cornejo, A., Rodríguez-García, M., 2009. Effect of temperature and steeping time on calcium and phosphorus content in nixtamalized corn flours obtained by the traditional nixtamalization process. Cereal Chem. 86 (5), 516–521.

Ruiz-Gutiérrez, M.G., Quintero-Ramos, A., Meléndez-Pizarro, C.O., Lardizábal-Gutiérrez, Barnard, J., Márquez-Melendez, R., Talamás-Abbud, R., 2010. Changes in mass transfer, thermal and physicochemical properties during nixtamalization of corn with and without agitation at different temperatures. J. Food Eng. 98, 76–83.

Safavi, K., Nakayama, T., 2000. Influence of mixing vehicle on dissociation of calcium hydroxide in solution. J. Endod. 26, 649–651.

Saleh, Z.S., Stanley, R., Wibisono, R., 2006. Separation and concentration of health compounds by membrane filtration. Int. J. Food Eng. 2 (3), Article 2004.

Salmeron-Alcocer, A., Rodriguez-Mendoza, N., Pineda-Santiago, S., Cristiani-Urbina, E., Juarez-Ramirez, C., Ruiz-Ordaz, N., Galindez-Mayer, J., 2003. Aerobic treatment of maize processing wastewater (nejayote) in a single stream multistage reactor. J. Environ. Eng. Sci. 2, 401–406.

Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods Enzymol. 299, 152–178.

Sotto, A., Arsuaga, J.M., Van der Bruggen, B., 2013. Sorption of phenolic compounds on NF/RO membrane surfaces: influence on membrane performance. Desalination 309, 64–74.

Strathmann, H., Giorno, L., Drioli, E., 2006. An introduction to Membrane Science and Technology. Consiglio Nazionale delle Richerche, Rome, Italy.

Todisco, S., Peña, L., Drioli, E., Tallarico, P., 1996. Analysis of the fouling mechanism in microfiltration of orange juice. Journal of Food Processing and Preservation 20, 453–466.

Valderrama-Bravo, C., Gutiérrez-Cortez, E., Contreras-Padilla, M., Rojas-Molina, I., Mosquera, J.C., Rojas-Molina, A., Beristain, F., Rodríguez-García, M.E., 2012. Constant pressure filtration of lime water (nejayote) used to cook kernels in maize processing. J. Food Eng. 110, 478–486.

Velasco-Martínez, M., Angulo, O., Vázquez-Couturier, D.L., Arroyo-Lara, A., Monroy-Rivera, J.A., 1997. Effect of dried solids of nejayote on broiler growth. Poult. Sci. 76 (11), 1531–1534.